

FUSARIA ASSOCIATED WITH MIMOSA WILT, SUMAC WILT, AND PINE PITCH CANKER¹

By WILLIAM C. SNYDER,² *associate professor of plant pathology, University of California*; E. RICHARD TOOLE, *associate pathologist*, and GEORGE H. HEPTING, *senior pathologist, Division of Forest Pathology*,³ *Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In recent years several new tree diseases caused by species of *Fusarium* have been described. At times other *Fusaria* in addition to the species responsible for the diseases have been found closely associated with the pathogens. The Asheville, N. C., laboratory of the Division of Forest Pathology has worked with 6 of the 10 species of *Fusarium* recognized by Snyder and Hansen (13, 14, 16)⁴ and with a number of formae in connection with studies on the mimosa wilt (5, 18), the sumac wilt (20), and the pitch canker disease of pine (8). This paper is intended to clarify the taxonomy and nomenclature of the *Fusaria* encountered in studying these 3 diseases and presents information of value in making identifications and in understanding the variability encountered in natural clones⁵ and laboratory mutants within a given species.

In the final assignment of names to the various species studied in this work, the system of classification and nomenclature of Snyder and Hansen (13, 14, 16) is used. In this system the species is based upon morphology and the forma in a trinomial denotes pathogenicity to a certain host or hosts. The extreme variability within some species, and even within some formae, with regard to spore size, pigmentation, conidial formation and septation, and gross cultural appearance among natural clones as well as subsequent mutants and among cultures exposed to different environmental conditions requires adoption of such a system if an endless number of species names—one applied to each variant—is to be avoided. Since this wide range of variation can be obtained among clones from single-spore cultures derived from a single pure culture of an isolate from nature, the naming of such

¹ Received for publication August 13, 1948.

² On temporary assignment to Division of Forest Pathology.

³ In cooperation with Southeastern Forest Experiment Station, U. S. Forest Service, Asheville, N. C.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 381.

⁵ The term "natural clone" denotes any form isolated directly from nature and is synonymous with Miller's "wild type" (9) where the latter is used in this sense. The term is used in opposition to "laboratory clone," the latter denoting mutants or growth types which develop in the course of laboratory culture practice.

variants as separate species is untenable. No spore measurements for the three formae being named are given in this paper, because their spore dimensions fall within the common ranges given by Wollenweber and Reinking (22) for the respective species.

IDENTIFICATION IN FUSARIUM

Species identification of an unknown *Fusarium* required that its morphologic features be determined, since these should constitute the sole basis on which the species are recognized. Since the sporulation of a *Fusarium* in nature is usually in the form of sporodochia, the attempt to determine *Fusarium* species directly from field specimens necessarily restricts a morphologic study largely to the sporodochia themselves. Often the macroconidia so obtained in nature reflect unfavorable influences of environment, substrate, other competitive organisms, and undermaturity or overmaturity. Furthermore, in nature more than one *Fusarium* species may invade a given substrate and form reproductive organs on the same tissues. Because of these facts, it is imperative that all efforts to identify *Fusarium* species be preceded by their transfer to a suitable culture medium by the single-spore method and that they be cultivated under conditions best suited to the development of the reproductive structures used in taxonomy.

PURE-CULTURE METHOD

Every culture referred to in this paper was initiated by a single spore transferred to a potato-dextrose-agar slant. Single-spore cultures were prepared either directly from spores found in nature, for example, on bark, or from conidia produced after a few days on tissue platings made from invaded plant parts. Ordinarily 10 single-spore cultures were prepared from each specimen; but, if the supply of conidia was inadequate, single-hyphal-tip cultures were prepared instead. Dilution plates of spores and of hyphal fragments were made on water agar.

During the summer and fall the cultures were laid on tables near a window, where they were exposed during their entire development to diffuse but not direct sunlight. Under these conditions most of the *Fusaria* included in this study sporulated normally; however, in some cases it was necessary to incubate the cultures outdoors, where they were subject not only to fluctuating daylight but also to widely fluctuating temperatures (15). Only in this way, for example, was there obtained an abundance of well-developed sporodochia of the pitch canker *Fusarium* from pine.

THE FUSARIA ON ALBIZZIA

The demonstration, by Hepting (5) and Toole (18) in the United States and later by Campi (2) in Argentina, of a vascular fusarium wilt of the mimosa tree (*Albizzia julibrissin* Durazz.) and the description by Voronikhin (21) of what is probably the same disease in the

Soviet Union have made important the correct identification of the various species of *Fusarium*, some saprogenic, occurring on the mimosa tree.

NAMES PREVIOUSLY APPLIED TO THE WILT FUSARIUM

Culture studies and inoculations have demonstrated that the mimosa wilt *Fusarium* in the United States and Argentina is a form of *F. oxysporum*. In the following discussion the names that have correctly or mistakenly been applied to this fungus are taken up in order of their appearance in the literature.

Fusarium albizziae Voron. (*Nectria albizziae* Voron.)

In 1920, Voronikhin (21) described a disease of the mimosa tree, epidemic in the Soviet Union, that appears to agree in all respects with that later described in the United States by Hepting (5). Although Voronikhin demonstrated the presence of microconidia in the infected vessels and observed cushions of *Fusarium* macroconidia on the surface of shoots killed by the wilt, he apparently made no cultures of the fungus and no inoculations. Nor did Voronikhin present any evidence to show whether the *Fusarium* found on the bark was the same fungus as that seen in the vascular tissue or even whether it was pathogenic. Without proof of the connection between the surface *Fusarium*, which he described as *F. albizziae*, and his vascular pathogen, this name cannot be accepted as that of the causal agent of mimosa wilt. Voronikhin's descriptions indicate strongly that the bark *Fusarium* was not of the wilt (*oxysporum*) type.

Perithecia found on a branch of mimosa killed by wilt were described by Voronikhin as *Nectria albizziae*, on the basis of their association with the *Fusarium* also found fruiting on the bark. No proof of genetic relationship to the *Fusarium* was given, and therefore there is no certainty that the ascomycete was the same fungus. The perithecia were small and protruded in a group on a cushionlike stroma. No wilt *Fusaria* are known to produce perithecia, and thus additional doubt is attached to Voronikhin's conclusions about the connection of this fungus with the vascular parasite.

Fusarium perniciosum Hepting

Proof that the pathogen of the vascular wilt of mimosa is a *Fusarium* was first established by Hepting (5), who also noted the resemblance of the fungus he isolated to other vascular wilt *Fusaria*. Partly because of its selective pathogenicity for the mimosa tree and partly because of the short macroconidia in his cultures (mean length of 28 μ for three-septate spores) Hepting named his fungus *F. perniciosum*, in keeping with the Wollenweber system of *Fusarium* classification. Hepting (5) and Toole (18) also mentioned clones differing widely from the common one in spore size and septation and in gross cultural appearance. These clones are now recognized merely as morphologic variants of the wilt fungus.

Fusarium oxysporum f. *perniciosum* (Hepting) Toole

This name was first suggested in a footnote by Toole (18) in 1941, but it was not proposed.

Fusarium oxysporum f. *perniciosum* (Hepting) Snyder

In 1943 Campi (2) published a paper in which the trinomial *F. oxysporum* f. *perniciosum* (Hepting) Snyder was used without Snyder's knowledge. Although Campi cited Toole's paper, either she did not see his footnote or she did not consider Toole's trinomial as valid under the rules, which state that the name of a taxonomic group is not validly published unless it is definitely accepted by the author who publishes it.

Campi's trinomial, however, also comes into question, since she did not definitely propose the combination but stated that *F. perniciosum*, according to the latest classification of Snyder, would be called *F. oxysporum* Schlecht. f. *perniciosum* (Hepting) Snyder.

NAME ACCEPTED FOR THE WILT FUSARIUM

In view of the confusion which exists in the nomenclature of the mimosa wilt pathogen, it seems desirable that a name validly published and still in accord with the trinomial system of *Fusarium* taxonomy (13, 14, 16) be indicated for acceptance.

It is proposed that the name *F. perniciosum* be changed, as suggested by Toole (18), in conformance with the *Fusarium* classification of Snyder and Hansen (13) as follows:

Fusarium oxysporum Schlecht. emend. Snyder and Hansen forma *perniciosum* (Hepting) Toole.

Syn. *F. perniciosum* Hepting (5).

F. oxysporum f. *perniciosum* (Hepting) Snyder (2).

A form of the species *F. oxysporum* pathogenic in the vascular tissues and causing a wilt in *Albizia julibrissin* and by inoculation in *A. lebeck* (L.) Benth., *A. lophantha* Benth., and *A. kalkora* (Roxb.) Prain (18). In southeastern United States, Argentina, and probably the Soviet Union.

ISOLATION OF FUSARIUM OXYSPORUM F. PERNICIOSUM

The mimosa wilt *Fusarium* may be obtained in culture by plating either the discolored vascular tissues of a diseased tree or the sporodochia which sometimes push out from the bark lenticels of a wilted tree when the environment is very humid. Sporodochia of some saprophytic *Fusaria*, however, occur even more commonly on the bark of dead or dying mimosa. Cultures made from sporodochia on the bark and from discolored wood beneath it have yielded identical clones of *F. oxysporum* f. *perniciosum* on several occasions, as shown by inoculation tests.

Hepting (5) found that several species of *Fusarium* may be recovered from mimosa bark and that at least one, which he recorded as *F. solani* var. *martii* (Appel and Wr.) Wr., could be isolated occasionally from diseased wood. This variety, synonymous with *F. solani* (Mart.) Appel and Wr. emend. Snyder and Hansen, was found to be unable to attack mimosa by itself (5); yet in nature it follows closely the wilt pathogen. This finding has recently been confirmed. Not only has *F. solani*, hidden in mixed cultures with *F. oxysporum* f. *perniciosum*,

been recovered from tissue platings of the discolored vascular region of wilted mimosa trees, but also the perfect stage, *Hypomyces solani* Reinke and Berth. emend. Snyder and Hansen, has been collected on the bark in association with sporodochia of *F. oxysporum* f. *perniciosum*. In fact, numbers of perithecia have been found in such cases clustered around sporodochia of the wilt *Fusarium*, an association which on the basis of appearances might well be considered to be that of a *Fusarium* and its *Nectria* or *Hypomyces* ascigerous stage. Only by making single-ascospore cultures from the perithecia and single-conidium cultures from the sporodochium in each case was the association detected. Perithecia of *Hypomyces* later developed in the single-ascospore cultures, especially in those incubated outdoors, demonstrating with certainty in these instances not only the identity of the fungus but also the fact that it was homothallic. Both heterothallic and homothallic strains of *H. solani* are known.

VARIABILITY IN *FUSARIUM OXYSPORUM* F. *PERNICIOSUM*

Although the use of natural clones is favored for the purpose of identification, it should be emphasized that natural clones may differ considerably among themselves (fig. 1). Some natural clones of the mimosa *Fusarium* produce abundant mycelium with but few slowly formed sporodochia; others form sporodochia rapidly and in abundance; still others are intermediate in appearance or differ in pigmentation from white to salmon to vinaceous shades or in their ability to develop sclerotia. Macroconidia may agree in size with those earlier described by Hepting for *F. perniciosum* (as in fig. 1, *C*), may be much larger (fig. 1, *D*) as in Hepting's Wisacky clone, or may otherwise reflect the variability in morphology displayed so commonly in the species *F. oxysporum*.

Selected natural clones, some of their variants, and their spores are shown for the mimosa wilt *Fusarium* in figure 1. The diverse clones shown here represent about 50 isolates made during the 1947 season, from different localities and States.

As an aid in recognizing the variability within this forma, the more common clones are described briefly as follows:

Clone A.—Commonest clone (fig. 1, *A*, last six tubes). Thallus raised; white, pink, or vinaceous shades; macroconidia rarely more than three-septate, produced abundantly in sporodochia and on surface of mycelium, salmon-colored in mass; microconidia abundant; no sclerotia, but often plectenchymic masses.

Clone B.—Hepting's Wisacky clone (fig. 1, *A*, middle three tubes). Thallus raised; white to vinaceous shades; macroconidia abundant, commonly four- or five-septate, produced in sporodochia and on surface of mycelium; microconidia abundant; blue-black sclerotia common.

Clone C.—Like clone *A*, but no sporodochia or plectenchymic masses (fig. 1, *A*, first three tubes, fig. 1, and *B*, fourth three tubes).

Clone D.—Pionnotal clone (fig. 1, *B*, first three tubes). No aerial mycelium; slimy spore mass over agar, mostly one- to three-septate macroconidia; some microconidia; color whitish to buff, pink or vinaceous; no sporodochia, plectenchymic masses, or sclerotia.

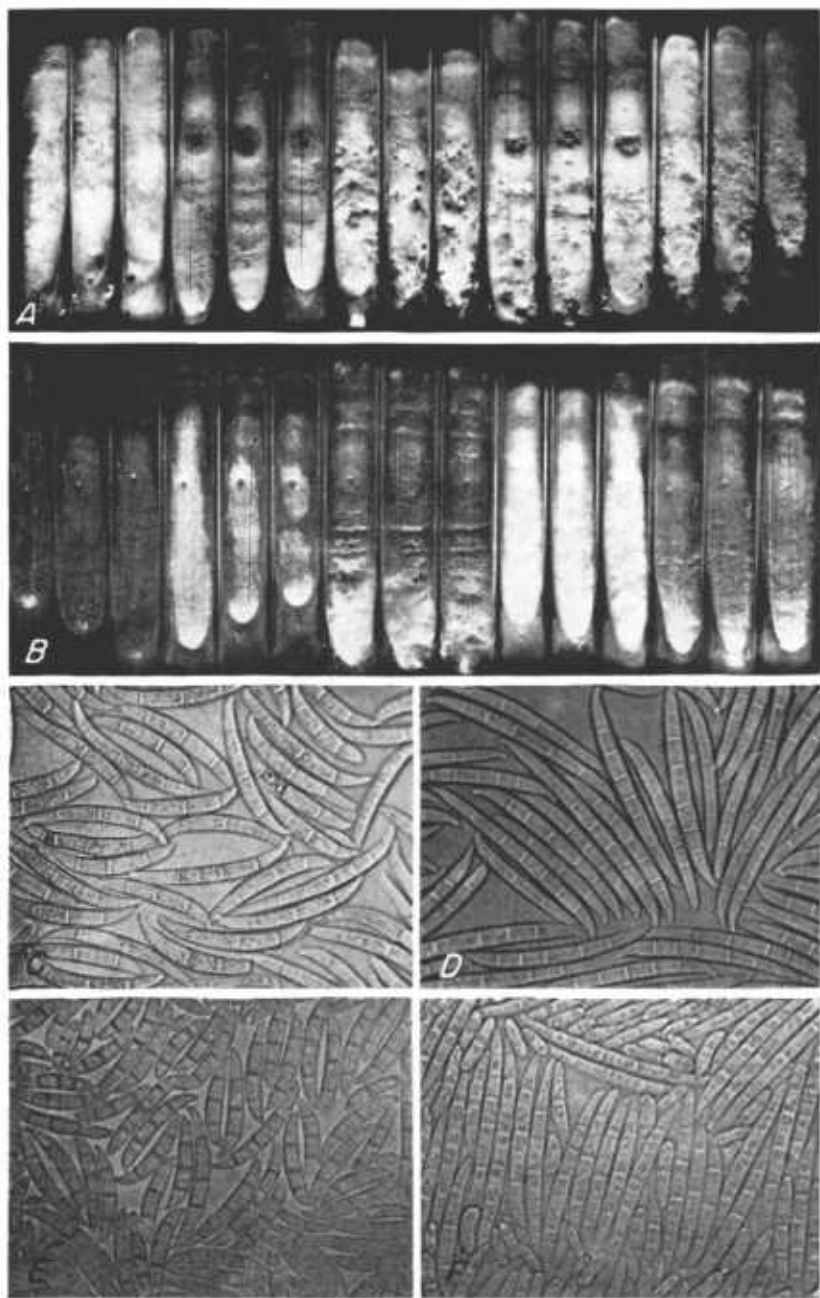


FIGURE 1.—*Fusarium oxysporum* f. *perniciosum*. A and B, Triplicate single-spore cultures of representative clones, made the same day on potato-dextrose agar: A, Identical with clones isolated directly from nature; B, laboratory mutants. C, Average-sized macroconidia from culture kept indoors. D, Long macroconidia from cultures made the same day from the same culture as those in C but kept outdoors. E, Very short macroconidia from a clone grown indoors. F, Long macroconidia from another clone grown indoors during the same period as those shown in C, D, and E. C-F, $\times 500$.

FUNGI ASSOCIATED WITH MIMOSA WILT

The Hyphomycetes and Ascomycetes commonly found on the bark of wilted mimosa in this investigation may be characterized briefly as follows:

Fusarium oxysporum f. *perniciosum*

Flesh-colored sporodochia, composed mostly of macroconidia, protruding from lenticels. In culture: pigmentation and cultural pattern various (see p. 370). Microconidia; macroconidia three- to five-septate, usually widest in the upper third; terminal and intercalary chlamydospores in the mycelium and spores.

Cultures of a form of *F. oxysporum* isolated from discolored xylem of a dying tree of *Albizzia procera* (Roxb.) Benth. in Puerto Rico failed to induce wilt in *A. julibrissin* in a small-scale test.

Fusarium solani (*Hypomyces solani*)

Cream, green, or blue (sometimes turning brown with age) sporodochia of macroconidia on bark or protruding from lenticels. In culture: cream, green, or blue pigmentation associated with spore masses, but mycelium usually not pigmented. Microconidia; terminal and intercalary chlamydospores in the mycelium and spores; macroconidia three- to five-septate or more, curved-cylindrical, apical cells usually blunt.

Ascigerous stage a *Hypomyces*; perithecia red, usually in groups around a sporodochium; ascospores hyaline, one-septate, usually tan-colored in mass.

Fusarium decemcellulare Brick (*Calonectria rigidiuscula* (Berk. and Br.) Sacc.)

Cream-colored spore masses inconspicuous, small, on bark. In culture: pigmentation next to agar usually carmine red, aerial mycelium white. Microconidia in chains, conspicuous; chlamydospores lacking; macroconidia in creamy masses, very large, 5- to 11-septate, curved-cylindrical as in *F. solani*.

The ascigerous stage was not observed on bark. Perithecia yellow to brown; ascospores hyaline, curved, usually three-septate, tan-colored in mass.

Fusarium episphaeria Snyder and Hansen (*Nectria episphaeria* Tode ex Fr.)

Conidial stage on bark inconspicuous or a flesh-colored slime. In culture: very slow-growing colonies, salmon to pink or orange-colored, consisting mostly of prostrate mycelium covered with a conidial slime, aerial mycelium usually absent. Microconidia usually absent, chlamydospores present or absent, macroconidia long, slender, one- to seven-septate.

Ascigerous stage in *Nectria*. Perithecia quite small, red, usually in groups on bark invaded by sphaeriaceous fungi; ascospores hyaline, one-septate, usually tan-colored in mass. (Not to be confused with another *Nectria* species which has similar perithecia, but an imperfect stage not in *Fusarium*, possibly in *Tuberularia*.)

Voronikhin's *F. albizziae* probably is a member of this species, and possibly his *Nectria albizziae* is also.

Fusarium lateritium Nees ex Fr. (*Gibberella lateritia* Snyder and Hansen)

Bright-pink to orange sporodochia, protruding from lenticels, consisting of macroconidia. In culture: pink, flesh, or orange sporodochia; mycelium white, pink, or variously colored, sometimes carmine red or white where in contact with the agar, colonies usually slower growing than those of *F. oxysporum* or *F. solani*, but faster growing than those of *F. episphaeria*. Microconidia and chlamydospores usually lacking. Macroconidia resembling those in *F. oxysporum* but usually longer, often with a tendency for the terminal cell to be hooked; three- to five-septate.

Ascigerous stage suspected on mimosa but not observed. Perithecia blue black, in groups; ascospores hyaline, curved, mostly three-septate, usually tan-colored in mass.

Fusarium roseum Lk. ex Fr. (*Gibberella rosea* Snyder and Hansen)

Bright-pink to flesh-colored sporodochia, often small but otherwise resembling those of *F. lateritium*. In culture: mycelium white, yellow, or red, very fast growing but often slow to produce conidia. Microconidia usually lacking; chlamydospores present or absent; macroconidia in flesh- to tan-colored sporodochia, apical cell not hooked.

Ascigerous stage not observed on mimosa. Perithecia blue black; ascospores hyaline, curved, usually three-septate, tan-colored in mass.

Tubercularia vulgaris Tode ex Fr. (*Nectria cinnabarina* Tode ex Fr.)

Conspicuous pink sporodochial cushions, protruding from lenticels, composed of microconidia. In culture: moderately scant white mycelium, producing pink to flesh-colored sporodochia of the *Tubercularia* stage in outdoor culture.

Ascigerous stage grouped around the sporodochia. Perithecia red, resembling those of *Hypomyces solani*; ascospores hyaline, one-septate. Observed at Washington, D. C., by Fowler and Stevenson (3).

Thyronectria austro-americana (Speg.) Seeler

Perithecia in clusters, light to dark brown; ascospores hyaline to pale straw-colored, three- to six-septate, muriform, often budding in the ascus and producing numerous ascoconidia.

Conidia of the *Gyrostroma* stage produced in pycnidia in the stroma, hyaline, nonseptate, orange-colored in mass.

Eutypa heteracantha Sacc.

Black perithecia grouped in a stroma, beaks protruding; ascospores hyaline, nonseptate. Fruiting commonly on dead bark of mimosa and frequently providing the substrate upon which *Nectria episphaeria* and *Nectria* sp. were found to develop.

THE SUMAC WILT FUSARIUM

During the summer of 1946 wilting and dying of staghorn sumac (*Rhus typhina* L.) were observed by G. H. Hepting along the Blue Ridge Parkway, near Waynesboro, Va. Examination of diseased plants disclosed that the disease was a vascular wilt, and cultures from the discolored xylem yielded a form of *Fusarium oxysporum*⁶ which has been shown to be pathogenic (20). Observations during the 1947 season were concerned principally with the extent of the infection in Virginia, inoculation tests, and taxonomic studies.

TAXONOMY AND NOMENCLATURE

Isolations of the causal *Fusarium* were made by tissue platings from the xylem of root, crown, trunk, branch, petiole, and base of the fruiting cluster of wilted plants. In each case, single-spore transfers were made directly from the sporulation on the bits of tissue. Also, numerous single-spore isolations were made directly from sporodochia found abundantly on the trunks and sometimes on the lower branches of wilted trees, especially where the sumac was located in moist or densely vegetated stands. In repeated isolations identical clones of a *Fusarium* were obtained from the sporodochia on the bark and the discolored xylem beneath it. These observations, together with inoculation data (20), showed that this wilt *Fusarium* can produce sporodochia on the bark, as in the case of the mimosa wilt fungus. *F. solani* was also isolated from the bark of diseased sumac, and its ascigerous stage, *Hypomyces solani*, was observed and identified with it culturally.

The sumac wilt *Fusarium* was typical of *F. oxysporum* in that, on potato-dextrose agar, it produced a white mycelial colony which later developed typical sporodochia. Microconidia developed abundantly and in false heads, intercalary and terminal chlamydospores were common in the mycelium and in old conidia, and the macroconidia were characteristic of the species (fig. 2).

⁶ Original isolations were made by R. W. Davidson, of the Division of Forest Pathology, United States Department of Agriculture, and original determination as *F. oxysporum* was made by H. N. Hansen and W. C. Snyder, of the Division of Plant Pathology, University of California.

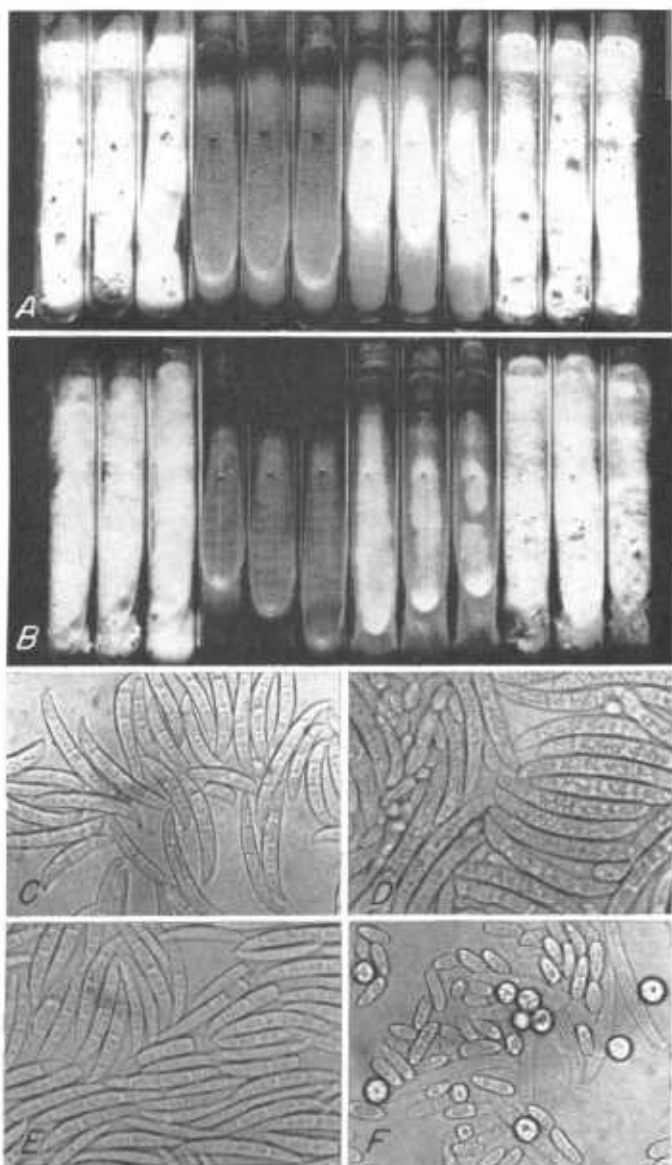


FIGURE 2.—*A*, Triplicate cultures of representative clones of *Fusarium oxysporum* f. *rhouis* (first and last three tubes, natural clones; third three, going into the pionnotal clone; and second three, pionnotal mutants). *B*, Clones of *F. oxysporum* f. *perniciosum* arranged below those in *A* to show their similarity to clones of *F. oxysporum* f. *rhouis*, when grown from single spores sown the same day on the same batch of medium. *C–F*, *F. oxysporum* f. *rhouis*: *C*, Small macroconidia produced indoors; *D*, large macroconidia and microconidia of the clone shown in *C* but produced outdoors; *E*, average-sized macroconidia; *F*, microconidia and chlamydospores mixed with a few macrospores. *C–F*, $\times 500$.

Single-spore clones of the sumac fungus have been found to belong to the species *F. oxysporum*, and these proved to be pathogenic to sumac. Since cross-inoculations with *F. oxysporum* f. *perniciosum*, the only other known vascular *Fusarium* pathogen of a tree, have been negative, the sumac pathogen is described here in conformity with the classification and nomenclature of Snyder and Hansen (13) as a new forma of this species. The following trinomial is proposed:

Fusarium oxysporum Schlecht. emend. Snyder and Hansen forma *rhois* Snyder and Hepting form. nov.

Parasitic in the vascular system and causing a wilt disease of *Rhus typhina* in the Blue Ridge Mountains of Virginia.⁷

VARIABILITY IN FUSARIUM OXYSPORUM F. RHOIS

The occurrence in nature of only one clone of this vascular pathogen is in sharp contrast to the mimosa wilt fungus, for which numerous natural clones have been established. The reason for this difference may be the fact that the sumac disease so far is known in only one small area, a few miles long. It seems possible that only one natural clone has established itself or has originated in this area, whichever the case may be. The mimosa wilt, on the other hand, is widespread throughout many States in the Southeast (19) and in a great variety of soil and climatic environments. Such a situation would be expected to result in time in the occurrence of geographical or ecological natural clones best suited to these different environments.

Although the isolates from nature so far have belonged to one clone, this clone, when cultivated in the laboratory in pure culture on potato-dextrose agar, has proved to be very unstable. Single-spore cultures of the natural clone, which is the typical sporodochial clone of *F. oxysporum*, have repeatedly yielded several mutants each succeeding time that they were single-spored. The most common mutant which continually arises from the sporodochial natural clone is the pionnotal one (fig. 2, A, second three tubes), and this may be considered as an expression of the Hansen dual phenomenon (4).

SIMILARITY BETWEEN THE SUMAC AND MIMOSA PATHOGENS

Although *F. oxysporum* f. *perniciosum* and *F. oxysporum* f. *rhois* are distinct biologically in that neither is pathogenic to the host of the other, their similarity in culture is very close. The natural clone of the sumac fungus may be matched in appearance closely with certain natural clones of the mimosa fungus. Furthermore, some of the mutants obtained from the mimosa fungus are almost identical in appearance with those from the sumac *Fusarium* (fig. 2, A and B), and no way has been found to tell them apart in their microscopic characters. The natural clone corresponds in appearance to clone B of *F. oxysporum* f. *perniciosum*, and it is known to throw mutants that agree with clones C and D of that fungus. These observations

⁷ The writers are indebted to E. K. Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, for her suggestion on the derivation of the forma name from *Rhus*.

further emphasize the basis for grouping them in the same species, yet keeping them separate on the basis of biological habit.

THE PINE PITCH CANCER FUSARIUM

The identity and characteristics of the *Fusarium* that causes the pitch canker disease of pine (8) are of particular interest (1) because this is the first disease of pines, other than seedling diseases, that has been ascribed to a *Fusarium* and (2) because of the value that this organism may have in prolonging gum flow from chipped pines in the naval stores industry (6). Whether the primary consideration may be the control of the pathogen responsible for pitch canker or the cultivation of, distribution of, and inoculation with a *Fusarium* beneficial in the production of turpentine and rosin, either the control or utilization of this organism depends upon an understanding of its identity and its behavior both in nature and in laboratory culture.

NATURAL DISTRIBUTION

Up to the present time the pitch canker fungus has been isolated from several species of southern pines in parts of Virginia, North Carolina, Tennessee, South Carolina, Georgia, Alabama, and Florida. Many natural cankers have been examined, especially in the North Carolina range of the disease, without finding fruiting of the *Fusarium*. This lack of fruiting of any kind has increased the difficulties in determining the kind of *Fusarium* involved. In old, inactive cankers the fungus is usually dead, and there is little promise of finding the fruiting stages on such material.

The only sporulation seen in the field on pines has been where the bark-covered wood of an active canker was artificially exposed or on the chipped surfaces of pines tapped for gum and artificially inoculated with the fungus. In these cases the *Fusarium* produces a light, white, patchy growth of mycelium on the wood itself. Later these patches often become pink and produce moderate amounts of conidia, most of which are microconidia but a few of which are macroconidia. This behavior, together with the fact that normal sporulation on undisturbed natural cankers has not been seen, suggests that the pine is not the only host of the *Fusarium*, but that under certain conditions the fungus has been able to establish itself on pine from inoculum perhaps produced on a very different host. Natural infection and distribution may take place through the aid of insects often found in connection with small new infections and observed to emerge from old cankers.

THE PINE FUSARIUM IN CULTURE

Since natural sporulation has been wanting, isolation of the pitch canker pathogen has depended upon the culture of bits of tissue taken from the wood and inner bark of active cankers (8). Single-spore isolations made directly from such tissue platings have given pure cultures of several natural clones.

During the 1947 season about 60 single-spore isolates of the *Fusarium* from pitch cankers in the aforementioned States and from 4 species of pine (*Pinus virginiana* Mill., *P. echinata* Mill., *P. caribaea* Mor., and *P. palustris* Mill.) were assembled. Most of these isolates

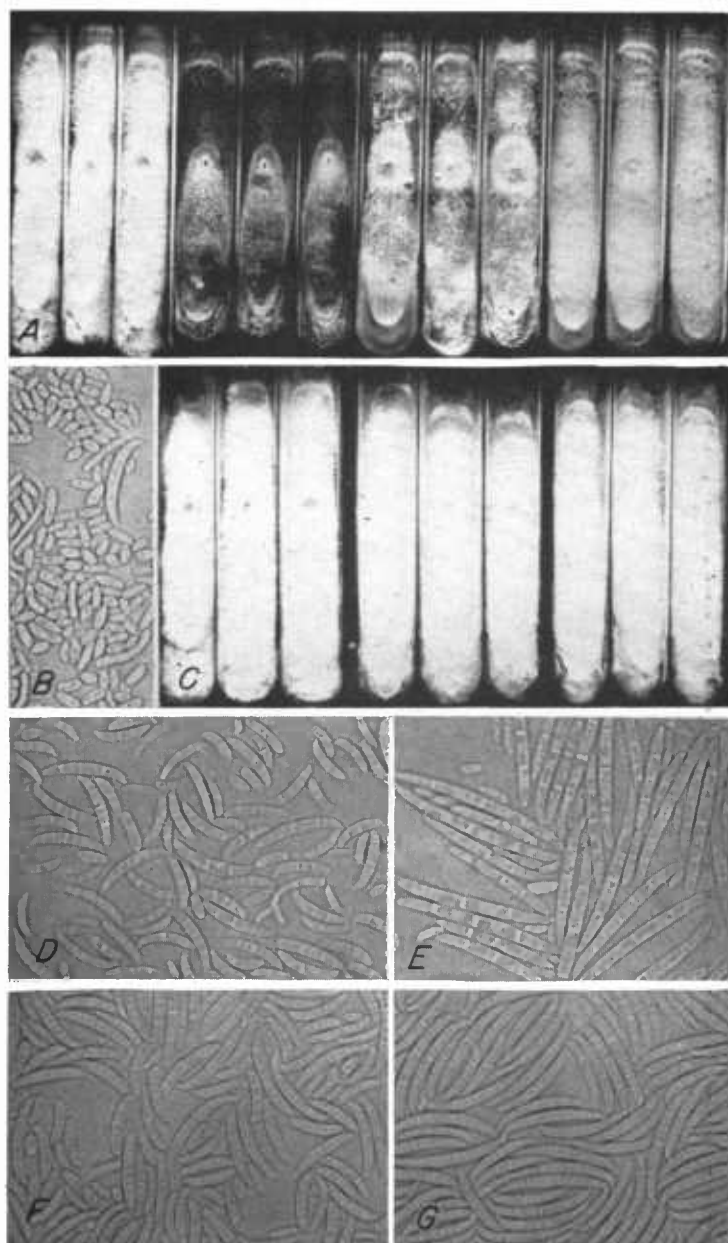


FIGURE 3.—*Fusarium lateritium* f. *pini*. A, Triplicate cultures of representative clones. B, Microconidia mixed with a few macrospores. C, Triplicate cultures of identical natural clones (type C) from *Pinus virginiana*, *P. palustris*, and *P. caribaea*, respectively. D-G, Macroconidia of different clones, all grown during the same period from single-spore cultures, showing great variation in size and shape. B and D-G, $\times 500$.

represent natural clones, but a few were laboratory mutants.⁸ These cultures provided the basis for the observations made on taxonomy and pathogenicity.

All natural clones in culture in the laboratory were characterized by a white fluffy mycelium which, in some clones, tended with age, to become pigmented in shades of blue, gray blue, green blue, and purple, especially in diffuse daylight. Many clones developed a purple pigment in the mycelium lying in contact with the potato-dextrose-agar slant. Some clones produced dark-bluish sclerotia. Many other natural clones differed in these and in other characters such as colony texture, configuration, zonation, and rate of growth (fig. 3, A).

Microscopically the laboratory-grown cultures were unsuited for taxonomic study, because macroconidia were almost entirely lacking even though the cultures were grown in light. In this absence of macroconidia the pine *Fusarium* could have fallen into the species *F. moniliforme*, into *F. oxysporum* if certain swellings in the mycelium were interpreted as chlamydospores, or into *F. lateritium*.

A good supply of normal macroconidia in sporodochia were produced on potato-dextrose cultures kept outdoors in the fall and subject to wide fluctuations in diffuse light and in temperature. Companion cultures of the same clones made at the same time on the same batch of medium but incubated in the laboratory near the window, produced no sporodochia.

The sporodochia of all natural clones grown outdoors were orange-colored, and the masses of macroconidia were often in sufficient abundance to give the entire slant an orange cast. The masses of macroconidia began to appear about a month after the cultures were placed outdoors, but they reached their greatest development in about 6 weeks.

Macroconidia were three- to five-septate, with a tendency toward the formation of a slight hook of the apical cell, characteristic of the small-spored types in *F. lateritium*. The apical-cell hook, the preponderance of macroconidia in optimum culture, and the absence of both chlamydospores and chain-formed microconidia place the fungus in *F. lateritium*. The fungus was first published under this name in 1947 (7).

The variation in cultural forms and in spore characteristics is evident in figure 3. Mutations from natural clones were occasionally obtained in the laboratory through single-spore transfer from single-spore cultures. The commonest mutant, recovered from several clones, was a pionnotal variant. Such cultures produced little aerial mycelium and a slime of spores on the surface of the agar and usually were deeper purple than the parent. The macroconidia of the pionnotal clones were generally much longer than those of the parent (fig. 3). Other mutants differed in the amount of pigmentation, amount and character of mycelium, and rate of growth.

⁸ Cultures and fresh canker material from Georgia and Florida were supplied by R. P. True and from Alabama by R. M. Lindgren, both of the Division of Forest Pathology.

TAXONOMY OF THE PINE FUSARIUM

After a study of cultures from sporodochial natural clones had established the fact that the pine *Fusarium* was *F. lateritium*, it was necessary to determine whether only clones of this species from pine would cause pitch canker and gum flow. To test this point, fresh isolates of a *F. lateritium* from *Albizzia* were used to make parallel inoculations in Virginia pine in conjunction with isolates of the pine form. Cankers and gum flow were obtained with isolates from pine only.

In view of the distinctive pathogenicity on pine of the isolates obtained from pitch cankers and the unique economic potentialities of this fungus in the naval stores industry, it is proposed to designate the pine *Fusarium* as a new form as follows:

F. lateritium Nees emend. Snyder and Hansen forma pini Hepting form. nov.

Pathogenic on the trunk, leader, and branches of pine (*Pinus virginiana*, *P. echinata*, *P. caribaea*, and *P. palustris*), causing thereon cankers characterized by heavy gum flow. Also prolongs gum flow from these hosts when applied to fresh wounds on them. Natural habitat, southeastern United States.

Clones grown on potato-dextrose agar in diffuse daylight include the following and various intermediate stages between them:

Clone A.—Common clone (fig. 3, A, first three tubes). Fast-growing, raised mycelial thallus, white through vinaceous shades, producing abundant microconidia in false heads indoors and abundant three- to five-septate macroconidia on sporodochia outdoors.

Clone B.—Sclerotial clone (fig. 3, A, third three tubes). Similar to A, but producing abundant sclerotia or blue-black plectenchyma.

Clone C.—Similar to clones A and B, but producing neither sporodochia nor sclerotia (fig. 3, A, fourth three tubes).

Clone D.—Pionnotal clone (fig. 3, A, second three tubes). No aerial mycelium. Macroconidia and microconidia in a slime over the agar. Whitish to vinaceous shades.

Clone E.—Very slow growing, bright purplish blue, producing mostly microconidia even outdoors, with semiraised mycelium.

The only other *Fusarium* isolated from pine wounds in this study was *F. roseum*. Both red and yellow-brown natural clones of this species were recovered. They failed to produce lesions when inoculated on *Pinus virginiana*. The *Gibberella rosea* stage was obtained in single-conidium cultures incubated outdoors during October and November.

DISCUSSION

In isolating clones of *Fusarium* species from nature, it is desirable that single-spore or single-hyphal-tip techniques be used either directly from the host or from the first tissue isolates. One of the most important reasons for this requirement is the tendency of all three pathogenic Fusaria herein described to live in close association with other Fusaria, notably *F. solani*, not only on the same host, but sometimes even in the same bark pustule or in the same vascular tissue. Natural clones were maintained through successive culturing by single-sporing all transfers and retaining those that were identical with the original clone.

The importance in identification of working with a large number of cultures transferred the same day on the same batch of medium was clearly shown by the varying effects of different environments on the appearance of cultures of parallel series during the 5 months in which these studies were carried on. For example, cultures of *F. lateritium* f. *pini* kept indoors in diffuse light produced abundant microconidia and practically no macroconidia, and such cultures would probably never have been identified as that species. Parallel cultures stored outdoors produced excellent sporodochia, abundant macroconidia, and almost no microconidia and were readily placed under *F. lateritium*. Cultures of *F. oxysporum* f. *perniciosum* kept indoors produced for the most part relatively short macroconidia, although some produced long macroconidia; whereas outdoor cultures produced macroconidia that were of average length for *F. oxysporum* generally. Thus some special handling, approaching more natural conditions (17), is often required to get good development of the structures used in identification, and the parallel study of many isolates is necessary to acquaint an investigator with the variations that may occur in a given species and forma.

The pine canker and mimosa wilt fungi illustrate in a striking way the multiplicity of morphologic clones of certain *Fusaria* in nature. These results, together with those of Borlaug (1) on flax wilt, Nelson, Coons, and Cochran (11) on celery wilt, and Snyder (12) on pea wilt, fail to substantiate Miller's (10) concept that usually there is only one "wild type," or natural clone, for a given *Fusarium*. The work just cited and the current studies on *F. oxysporum* f. *perniciosum* and *F. lateritium* f. *pini* show for several *Fusaria* wide variability in isolates made on the same medium directly from nature. Comparisons made by some workers of isolates of a given *Fusarium* obtained from a variety of sources over a wide range of years are not accepted by Miller as valid comparisons of natural clones, since some mutations undoubtedly took place in the meantime. In the case of Miller's muskmelon *Fusarium* in Canada (9) and of the sumac *Fusarium*, the occurrence of one predominating natural clone seems to be the rule. However, the variation in natural clones of the flax wilt, pea wilt, celery wilt, pine canker, and mimosa wilt *Fusaria*, in addition to the experience of the writers with other *Fusaria*, indicates that Miller's assertion (10) that one "wild type" predominates and that variation in types isolated directly from nature "must be relatively infrequent" should not be applied to *Fusaria* in general.

The morphological variability shown within the pathogenic formae of the mimosa wilt and pine canker *Fusaria* follows the pattern of other *Fusaria* (1, 9, 10, 11, 12, 13, 14, 16, 22) and poses important questions concerning the identification of such pathogens, procedures for the maintenance of stock cultures, selection of the clone for deposition in type-culture collections, and choice of clones for use in projects aimed at nomenclature, disease resistance, and control. Certainly an intelligent approach to the problems presented by these diseases requires recognition of the phenomenon of morphologic and physiologic variability demonstrated to occur in nature.

SUMMARY

Clones of *Fusaria* pathogenic to species of *Albizzia*, *Pinus*, and *Rhus*, isolated in pure culture from trees in numerous locations, provided the bases for the comparative studies on the cultural characteristics, taxonomy, and nomenclature of these fungi. Single-spore or single-hyphal-tip cultures made directly from the host or from fresh-tissue cultures were used throughout the work, both in the isolation of natural clones and in subsequent subculturing.

Although one or more clones of each of the three pathogens were shown to be variable in culture through mutation, two of the pathogens—those of *Albizzia* and of *Pinus*—were found to exist in nature in a highly variable state, each consisting of numerous distinct, morphologic clones.

The trinomial *Fusarium oxysporum* f. *perniciosum* (Hepting) Toole is accepted for the mimosa wilt pathogen; *F. oxysporum* f. *rhois* Snyder and Hepting is proposed for the sumac wilt pathogen; and *F. lateritium* f. *pini* Hepting is proposed for the pine pitch canker *Fusarium*.

Procedures applicable to pure-culture isolation, the maintenance of original clones, and the induction of optimum sporulation are outlined for each pathogen, and brief descriptions of clones are provided as a guide to their identification. Sporodochia of the pitch canker *Fusarium* were obtained only in outdoor culture under conditions of fluctuating light and temperature.

Consideration is given also to the saprophytes commonly associated with the above-named pathogens in diseased trees.

Some sporodochia on the bark of diseased mimosa and sumac yielded pure cultures of the pathogens, whereas others proved to be those of saprophytic *Fusaria*. No sporulation of the pitch canker *Fusarium* was observed on natural cankers in the field, but some occurred on chipped faces of pines artificially inoculated.

LITERATURE CITED

- (1) BORLAUG, N. E.
1945. VARIATION AND VARIABILITY OF FUSARIUM LINI. Minn. Agr. Expt. Sta. Tech. Bul. 168, 40 pp., illus.
- (2) CAMPI, M. D.
1943. EL MARCHITAMIENTO DE LA ACACIA DE CONSTANTINOPLA ("ALBIZZIA JULIBRISSIN") Y SU RELACIÓN CON LA PRESENCIA DE "FUSARIUM OXYSPORUM" SCHL. F. "PERNICIOSUM" (HEPTING) SNYDER. Lilloa 9: [457]-460, illus.
- (3) FOWLER, M. E., and STEVENSON, J. A.
1944. A CANKER AND SOME DECAY FUNGI ON MIMOSA. (Phytopath. note) Phytopathology 34: 985-987, illus.
- (4) HANSEN, H. N.
1938. THE DUAL PHENOMENON IN IMPERFECT FUNGI. Mycologia 30: 442-455, illus.
- (5) HEPTING, G. H.
1939. A VASCULAR WILT OF THE MIMOSA TREE (ALBIZZIA JULIBRISSIN). U. S. Dept. Agr. Cir. 535, 11 pp., illus.
- (6) ———
1947. STIMULATION OF OLEORESIN FLOW IN PINES BY A FUNGUS. Science 105: 209, illus.
- (7) ———
1947. STIMULATION OF GUM FLOW BY A FUNGUS. Forest Farmer 6 (6): 2.
- (8) ——— and ROTH, E. R.
1946. PITCH CANKER, A NEW DISEASE OF SOME SOUTHERN PINES. Jour. Forestry 44: 742-744, illus.
- (9) MILLER, J. J.
1945. STUDIES ON THE FUSARIUM OF MUSKMELON WILT. I. PATHOGENIC AND CULTURAL STUDIES WITH PARTICULAR REFERENCE TO THE CAUSE AND NATURE OF VARIATION IN THE CAUSAL ORGANISM. Canad. Jour. Res., Sect. C, 23: 16-43, illus.
- (10) ———
1946. CULTURAL AND TAXONOMIC STUDIES ON CERTAIN FUSARIA. I. MUTATION IN CULTURE. Canad. Jour. Res., Sect. C, 24: 188-212, illus.
- (11) NELSON, R., COONS, G. H., and COCHRAN, L. C.
1937. THE FUSARIUM YELLOW DISEASE OF CELERY . . . Mich. Agr. Expt. Sta. Tech. Bul. 155, 74 pp., illus.
- (12) SNYDER, W. C.
1933. VARIABILITY IN THE PEA-WILT ORGANISM, FUSARIUM ORTHOCERAS VAR. PISI. Jour. Agr. Res. 47: 65-88, illus.
- (13) ——— and HANSEN, H. N.
1940. THE SPECIES CONCEPT IN FUSARIUM. Amer. Jour. Bot. 27: 64-67.
- (14) ——— and HANSEN, H. N.
1941. THE SPECIES CONCEPT IN FUSARIUM WITH REFERENCE TO SECTION MARTIELLA. Amer. Jour. Bot. 28: 738-742.
- (15) ——— and HANSEN, H. N.
1941. THE EFFECT OF LIGHT ON TAXONOMIC CHARACTERS IN FUSARIUM. Mycologia 33: 580-591, illus.
- (16) ——— and HANSEN, H. N.
1945. THE SPECIES CONCEPT IN FUSARIUM WITH REFERENCE TO DISCOLOR AND OTHER SECTIONS. Amer. Jour. Bot. 32: 657-666.
- (17) ——— and HANSEN, H. N.
1947. ADVANTAGES OF NATURAL MEDIA AND ENVIRONMENTS IN THE CULTURE OF FUNGI. (Phytopath. note) Phytopathology 37: 420-421.
- (18) TOOLE, E. R.
1941. FUSARIUM WILT OF THE MIMOSA TREE (ALBIZZIA JULIBRISSIN). Phytopathology 31: 599-616, illus.
- (19) ———
1948. DISTRIBUTION OF MIMOSA WILT IN 1947. U. S. Bur. Plant Indus., Soils, and Agr. Engin., Plant Dis. Rptr. 32: 67, illus. [Processed]
- (20) ——— SNYDER, W. C., and HEPTING, G. H.
1948. A NEW FUSARIUM WILT OF SUMAC. (Abstract) Phytopathology 38: 572.

- (21) VORONIKHIN [WORONICHIN], N. N.

1920. FUSARIUM ALBIZZIAE N. SP., CAUSE PROBABLE DU DÉPÉRISSEMENT D'ALBIZZIA JULIBRISSIN BOISS. DANS L'ARRONDISSEMENT DE BATUM (communication préliminaire). Vest. Tiflis. Bot. Sada (Monit. Jard. Bot. de Tiflis) 48: [15]-36, illus. [In Russian. French summary, pp. 34-36.]

- (22) WOLLENWEBER, H. W., and REINKING, O. A.

1935. DIE FUSARIEN: IHRE BESCHREIBUNG, SCHADWIRKUNG UND BEKÄMPFUNG. 355 pp., illus. Berlin.